3 SCREENING FOR DRUGS USING THE ABBOTT AXSYM ASSAY

3.1 Summary

3.1.1 The Axsym assay is an automated semi-quantitative reagent system designed for the detection of drugs of abuse (DOA) in urine and a quantitative reagent system designed for the measurement of acetaminophen and salicylate in serum and plasma. The DOA assays, utilizing Fluorescence Polarization Immunoassay (FPIA) technology, have been modified for use with whole blood samples, fluids and tissue homogenates extracted with acetonitrile. The acetaminophen and salicylate assays, utilizing FPIA technology, have been modified for use with whole blood samples, fluids and tissue homogenates diluted with buffer. The samples are run with Axsym Controls and spiked blood sample controls. The results obtained are presumptive, meaning that any "positive" result requires appropriate confirmation.

3.2 Specimen Requirements

3.2.1 Approximately 1 mL of whole blood, fluid(s) or tissue dilutions/homogenates.

3.3 Reagents and Standards

3.3.1 Abbott Axsym System Reagent Packs: Acetaminophen, Salicylate, Amphetamine/Methamphetamine II, Barbiturates IIu, Benzodiazepines, Benzoylecgonine, Cannabinoids, Opiates, Phencyclidine II

3.3.2 Acetonitrile

3.3.3 Axsym Systems Solution 4 Line Diluent

3.3.4 Hydrocodone, 1 mg/mL

3.3.5 Alprazolam, 1 mg/mL

3.3.6 Phencyclidine, 1 mg/mL

3.3.7 Benzoylecgonine, 1 mg/mL

3.3.8 Butalbital, 1 mg/mL

3.3.9 9-Carboxy-11-nor-delta 9-THC, 1 mg/mL

3.3.10 Acetaminophen, 1 mg/mL

3.3.11 Sodium salicylate

3.4 Solutions, Internal Standards, Calibrators, Controls

3.4.1 Abbott Systems Multiconstituent Control, Drugs of Abuse (DOA)

3.4.2 Abbott Systems Acetaminophen Control

3.4.3 Abbott Systems Salicylate Control

3.4.4 Abbott System Calibrators: Cannabinoids, Phencyclidine II,, Acetaminophen, Salicylate, Benzoylecgonine, Amphetamine/Methamphetamine II, Benzodiazepines, Barbiturates IIu, Opiates

3.4.5 DOA Control Stock Solution
Add the following volumes to a 25 mL volumetric flask and QS to volume with methanol:

	Drug	µL of 1 mg/mL standard	Final concentration (mg/L)
3.4.5.1	Hydrocodone	25	1
3.4.5.2	Alprazolam	25	1
3.4.5.3	Phencyclidine	25	1
3.4.5.4	Benzoylecgonine	125	5
3.4.5.5	Butalbital	250	10
3.4.5.6	THCA	10	0.4

3.4.6 DOA Spiked Blood Control

3.4.6.1 Add 0.1 mL of the Control Stock Solution to 1 mL of blank blood (blood bank blood previously determined not to contain drugs) to provide target values as follows (in mg/L):

3.4.6.1.1	Hydrocodone	0.1
3.4.6.1.2	Alprazolam	0.1
3.4.6.1.3	Phencyclidine	0.1
3.4.6.1.4	Benzoylecgonine	0.5
3.4.6.1.5	Butalbital	1.0
3.4.6.1.6	THCA	0.04

3.4.7 Acetaminophen stock solution (0.1 mg/mL): Pipet 200 µL of the 1 mg/mL acetaminophen standard into a 2 mL volumetric flask and QS to volume with dH_2O.

3.4.8 Acetaminophen blood control: Add 100 µL of the 0.1 mg/mL acetaminophen stock solution to 1 mL blank blood for a final concentration of 10 mg/L.

3.4.9 Salicylate working solution (0.5 mg/mL): Weigh 58 mg of sodium salicylate and transfer to a 100 mL volumetric flask. QS to volume with dH_2O.

3.4.10 Salicylate blood control: Add 100 µL of the 0.5 mg/mL salicylate working solution to 1 mL blank blood for a final concentration of 50 mg/L.

3.4.11 Blank blood control (negative control). Blood bank blood previously determined not to contain drugs.

3.5 Apparatus

3.5.1 Test tubes, 13 x 100 mm disposable glass

3.5.2 Vortex mixer

3.5.3 Centrifuge capable of 2,000-3,000 rpm

3.5.4 Abbott Axsym System

3.6 Procedure

3.6.1 Drugs of Abuse Assays

3.6.1.1 Label 13 mm x 100 mm glass disposable test tubes appropriately, blood blank, spiked blood control and case sample IDs.

3.6.1.2 Prepare DOA spiked blood control.

3.6.1.3 Add 1 mL case specimens to the appropriately labeled tubes.

3.6.1.4 Slowly, add dropwise 2 mL COLD (freezer temperature) acetonitrile to each tube while vortexing. Continuous vortexing, not mere mixing, is essential. Continue vortexing for an additional 30 seconds.

3.6.1.5 Centrifuge at approximately 3000 rpm for 15 minutes.

3.6.1.6 Place tubes in refrigerator for at least 2 hours.

3.6.1.7 Just prior to loading samples onto Axsym, place samples in freezer for 15 minutes to ensure separation into 3 layers. Bottom - blood clot, Middle - serum, Top - acetonitrile.

3.6.1.8 Perform all daily start-up procedures according to the Axsym System Operations Manual and record in the Maintenance Log. (Perform weekly, monthly, quarterly, and other maintenance specified in the Operations manual as required).

3.6.1.9 Order controls and samples to Orderlist. Print Orderlist.

3.6.1.10 Pipet approximately 350 µl of Abbott Systems Multiconstituent Low, Med or High Control into the first sample cup of Tube Segment A.

3.6.1.11 Transfer the top acetonitrile layer from each sample, blank blood control and spiked blood control into the appropriate sample tube, according to Orderlist.

3.6.1.12 Load the Tube Segments and reagent packs onto the instrument, and press "RUN". When the run is complete the instrument displays a “Scan Reagent Pack” pop up box, follow instructions displayed on screen. If reagent packs are removed, press OK.

3.6.2 Acetaminophen and Salicylate Assays

3.6.2.1 Prepare acetaminophen and salicylate spiked blood controls.

3.6.2.2 Perform all daily start-up procedures according to the Axsym System Operations Manual and record in the Maintenance Log. (Perform weekly, monthly, quarterly, and other maintenance specified in the Operations manual as required).

3.6.2.3 Order controls and samples to Orderlist. Print Orderlist.

3.6.2.4 Pipet approximately 100 µl of Abbott Systems Acetaminophen Control and Abbott Systems Salicylate Control into the first two sample cups of Tube Segment A.

3.6.2.5 Pipet 200 µL Axsym Systems Solution 4 Line Diluent into each sample cup. Pipet 100 µL of case samples, blank blood and spiked blood controls into the appropriate sample cups for a 1:3 dilution, mixing the sample with the buffer while pipetting. Remove any air bubbles.

3.6.2.6 Load the Tube Segments and reagent packs onto the instrument, and press "RUN". When the run is complete the instrument displays a “Scan Reagent Pack” pop up box, follow instructions displayed on screen. If reagent packs are removed, press OK.

3.7 Calculation

3.7.1 Release results to print Sample Reports. Print Controls, record QC and file results in the Axsym QC book. A positive result must have a quantitative value equal to or greater than the cut-off, which is listed on the Sample Report.

3.7.2 Record results on case file worksheets and place each Sample Report in the case file.

3.8 Quality Control and Reporting

3.8.1 The Abbott Systems Multiconstituent Control is run at the beginning of each DOA run and recorded in the QA/QC logbook.

3.8.2 The Abbott Systems Acetaminophen and Salicylate controls are run at the beginning of the acetaminophen/salicylate run are recorded in the QA/AC logbook.

3.8.3 Blank blood and spiked blood controls are run with each batch and recorded in the QA/QC logbook.

3.8.4 When confirmed appropriately, quantitative acetaminophen and salicylate results may be reported.

3.9 Notes

3.9.1 The laboratory must have data establishing cutoffs for each immunoassay. Cutoffs are established by spiking negative blood samples with a series of drug concentrations near the desired limit of detection. By comparing results of known negative blood and the series of concentrations, a cutoff is established. The cutoff is validated by reanalyzing samples previously quantitated by GCMS. In addition, cutoffs may be adjusted by monitoring positive screen results versus GCMS quantitations and confirmations to reduce false positives and false negatives. Occasionally, with significant changes in reagents (new tracer pool, antiserum, lot number), cutoffs may have to be reestablished.

3.9.2 Each FPIA assay has different cross-reactivities with drugs within the same class, so cutoffs need to take into consideration therapeutic levels of drugs as well as their cross reactivity with the reagents.

3.9.3 The cutoffs were established to detect the following concentration of drugs in whole blood samples:

FPIA Assay	Target Analytes	Blood Concentration (mg/L)
Amphetamine/ Methamphetamine II	Amphetamine	0.05
	Methamphetamine	0.05
	3,4-Methylenedioxymethamphetamine	0.05
	Methylenedioxyamphetamine	0.05
Barbiturates IIu	Amobarbital	0.5
	Butabarbital	0.5
	Butalbital	0.5
	Pentobarbital	0.5
	Phenobarbital	0.5
	Secobarbital	0.5
Benzoylecgonine	Benzoylecgonine	0.10
Benzodiazepines	Alprazolam	0.02
	Chlordiazepoxide	0.2
	Clonazepam	0.02
	Diazepam	0.1
	Lorazepam	0.02
	n-Desalkylflurazepam	0.02
	Nordiazepam	0.1
	Oxazepam	0.1
	Temazepam	0.02
Cannabinoids	THC carboxylic acid	0.005
Opiates	Codeine	0.02
	Hydrocodone	0.02
	Morphine	0.02
	6-Acetylmorphine	0.01
	Oxycodone	0.02
Phencyclidine II	Phencyclidine	0.01

3.10 References

3.10.1 Abbott Axsym System Operations Manuel 69-4606/R6, February 2000, Abbott Diagnostics, Abbott Park, IL.